PEPTIDE DEFORMYLASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to the use of novel *N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-arylamide compounds, and pharmaceutical compositions containing these compounds as peptide deformylase inhibitors.

BACKGROUND OF THE INVENTION

Bacterial initiator methionyl tRNA is modified by methionyl tRNA formyltransferase (FMT) to produce formyl-methionyl tRNA. The formyl methionine (f-Met) is then incorporated at the N-termini of newly synthesized polypeptides. Polypeptide deformylase (PDF or Def) then deformylates primary translation products to produce N-methionyl polypeptides. Most intracellular proteins are further processed by methionine amino peptidase (MAP) to yield the mature peptide and free methionine, which is recycled. PDF and MAP are both essential for bacterial growth, and PDF is required for MAP activity. This series of reactions is referred to as the methionine cycle (Figure 1)

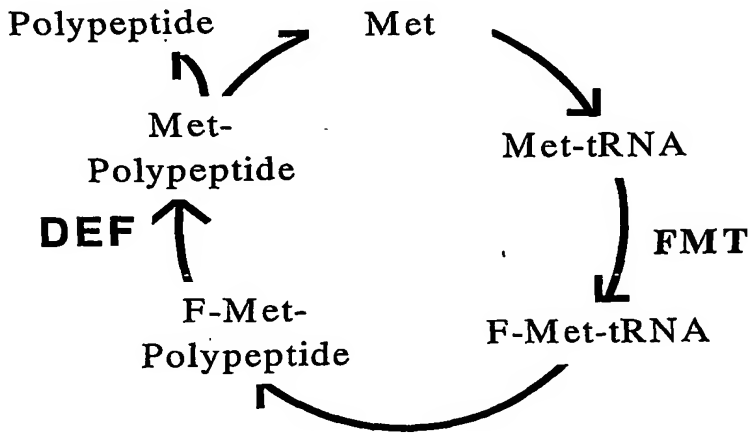


Figure 1. The methionine cycle.

To date, polypeptide deformylase homologous genes have been found in bacteria, in chloroplast-containing plants, in mice and in human. The plant proteins are nuclear encoded but appear to carry a chloroplast localization signal. This is consistent with the observation that chloroplast RNA and protein synthesis processes are highly similar to those of eubacteria. No information on protein expression of mammalian PDF gene homologs or functional role for such proteins has been demonstrated to date (Meinnel T. 2000, Parasitology Today, 16(4), 165-168).

Polypeptide deformylase is found in all eubacteria for which high coverage genomic sequence information is available. Sequence diversity among PDF homologs is high, with as little as 20% identity between distantly related sequences. However, conservation around the active site is very high, with several completely conserved residues, including one cysteine and two histidines which are required to coordinate the active site metal (Meinnel, T. et al, 1997, Journal of Molecular Biology, 267, 749-761).

PDF is recognized to be an attractive anti-bacterial target, as this enzyme has been demonstrated to be essential for bacterial growth in vitro (Mazel, D. et al, EMBO J. 13 (4), 914-923, 1994), is not involved in eukaryotic protein synthesis (Rajagopalan et al, J. Am. Chem. Soc. 119, 12418-12419, 1997), and is universally conserved in prokaryotes (Kozak, M. Microbiol. Rev. 47, 1-45, 1983). Therefore PDF inhibitors can potentially serve as broad spectrum anti-bacterial agents.

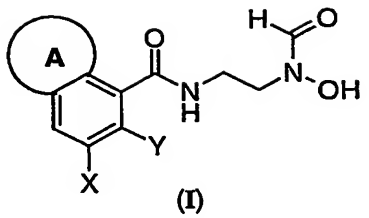
SUMMARY OF THE INVENTION

The present invention involves novel *N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-arylamides with bacterial polypeptide deformylase inhibiting activity represented by Formula (I) hereinbelow and their use as PDF inhibitors.

The present invention further provides methods for inhibiting PDF in an animal, including humans, which comprises administering to a subject in need of treatment an effective amount of a compound of Formula (I) as indicated hereinbelow.

DETAILED DESCRIPTION OF THE INVENTION

The compounds useful in the present methods are selected from Formula (I) hereinbelow:



(I)

wherein:

A is a fused aromatic or aliphatic ring system consisting of five to seven atoms and incorporating zero to four heteroatoms, such that **A** may be optionally substituted with one, two, or three substituents selected from the group consisting of: optionally substituted alkyl or cycloalkyl of one to nine carbons, halo, alkoxy of one to nine carbons, hydroxy, amino, hydroxyalkyl of one to nine carbons, alkoxyalkyl, optionally substituted aryl or optionally substituted heteroaryl, carboxy, and alkoxycarbonyl, and **X** and **Y** are, independently, halo, hydroxy, or hydroxyalkyl of one to three carbons.

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined together by carbon-carbon bonds. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated.

As used herein, "aryl" refers to an optionally substituted aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. "Aryl" includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

Preferred compounds of the present invention include:

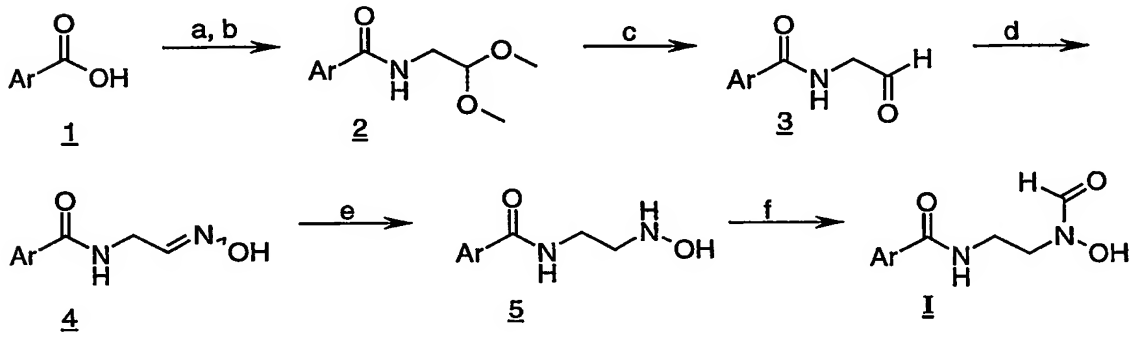
5-Chloro-4-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]amido-benzimidazole.

Also included in the present invention are pharmaceutically acceptable salts and complexes. Preferred are the hydrochloride, hydrobromide and trifluoroacetate salts. The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

The compounds and processes of the present invention will be better understood in connection with the following synthetic schemes, which are merely illustrative of the methods by which the compounds of the invention may be prepared and are not intended to limit the scope of the invention as defined in the appended claims.

Compounds of the formula (I) may be prepared according to the Scheme 1.

Scheme 1



a) $(COCl)_2$, DMF, DCM; b) aminoacetaldehyde dimethyl acetal, TEA, DCM 0°C; c) THF, 6N HCl; d) $NH_2OH \cdot HCl$, NaOAc, MeOH; e) $NaCNBH_3$, HCl, MeOH, 0°C; f) $HCO_2C(O)CH_3$, pyridine, 0°C.

Aryl carboxylic acids (1) may be purchased or prepared by standard literature procedures. Conversion of (1) to the acid chloride and amination with the dimethyl acetal of aminoacetaldehyde provides the amide (2). Deprotection with 6NHCl in THF and treatment of the resulting aldehyde (3) with hydroxylamine and sodium acetate in MeOH provides the oxime (4). Reduction of the oxime to the hydroxylamine (5) is accomplished with $NaCNBH_3$ in MeOH under acidic conditions. Finally, *N*-formyl-*N*-hydroxylamine (I) is obtained by treatment of the hydroxylamine in pyridine with the mixed anhydride formed from formic acid and acetic anhydride.

SB-751556, 5-Chloro-4-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]amido-benzimidazole was prepared from 5-chloro-4-carboxy-benzimidazole in a similar manner to the above examples.

Preparation of 5-chloro-4-carboxy-benzimidazole

a) 6-amino-2-chloro-5-nitrobenzoic acid

A mixture of 2,6-dichloro-3-nitrobenzoic acid (2.93 g, 12.4 mmol), copper (I) chloride (0.025 g, 0.25 mmol), and aqueous ammonium chloride (12.5 mL) were stirred in a sealed vessel at 125°C for 18 h. The mixture was acidified with 6N HCl. The yellow solid precipitate was washed with 6N HCl and dried to provide the title compound as a mixture with 6-amino-2-chloro-3-nitrobenzoic acid. ^{1}H NMR* (400 MHz, CD_3OD): δ 8.17 (d, j = 9.2 Hz, 1H); 6.78 (d, j =9.2 Hz, 1H). * major isomer

b) methyl 6-amino-2-chloro-5-nitrobenzoate

(Trimethylsilyl)diazomethane (2M in hexanes, 8 mL, 16 mmol) was added dropwise to a mixture of 6-amino-2-chloro-5-nitrobenzoic acid and 6-amino-2-chloro-3-nitrobenzoic acid (1.74 g, 8.03 mmol) in dichloromethane (52 mL) and methanol (17 mL) at 0°C. The mixture was stirred 30 min. and was concentrated on a rotary evaporator. The residue was purified by flash column chromatography (silica gel, 30% ethyl acetate/hexanes) to provide the title compound as a yellow solid (0.85 g, 46%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.02 (d, j = 9.2 Hz, 1H); 6.61 (d, j = 9.2 Hz, 1H); 3.81 (s, 3H).

c) methyl 2-chloro-5,6-diamino-benzoate

Methyl 6-amino-2-chloro-5-nitrobenzoate (0.85 g, 3.69 mmol) and tin (II) chloride diazomethane (3.5 g, 18.4 mmol) were refluxed in methanol (25 mL) for 2 h. The mixture was cooled to room temperature and was concentrated on a rotary evaporator. The residue was partitioned between ethyl acetate and saturated aqueous potassium hydroxide. The aqueous phase was extracted with ethyl acetate (3 X). The combined organic extracts were dried ($MgSO_4$) and were evaporated to provide the

title compound as a brown oil (0.73 g, 97%). ^{1}H NMR (400 MHz, CD_3OD): δ 6.54 (d, j = 8.3 Hz, 1H); 6.42 (d, j = 8.3 Hz, 1H); 3.74 (s, 3H).

d) 4-methoxycarbonyl-5-chlorobenzimidazole

Methyl 2-chloro-5,6-diamino-benzoate (0.73 g, 3.61 mmol) was refluxed in formic acid (30 mL) for 18 h. The mixture was cooled to room temperature and was concentrated on a rotary evaporator. The residue was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The aqueous phase was extracted with ethyl acetate (3 X). The combined organic extracts were dried ($MgSO_4$). The residue was triturated from diethyl ether/hexanes to provide the title compound as a tan solid (0.47 g, 60%). ESMS: M+H = 211.

e) 5-chloro-4-carboxy-benzimidazole

4-methoxycarbonyl-5-chlorobenzimidazole (0.46 g; 2.18 mmol) and sodium hydroxide (0.43 g, 11 mmol) were refluxed in tetrahydrofuran (5 mL), methanol (5 mL) and water (2 mL) for 18 h.formic acid (30 mL) for 18 h. The mixture was cooled and was acidified with 1N HCl. The mixture was washed with ethyl acetate (2 X) and the water evaporated *in vacuo*. The solid residue was stirred in methanol (20 mL) and was filtered. The filtrate was evaporated to provide the title compound as a tan solid (100%). ESMS: M+H = 197.

The following compounds can be prepared in a similar manner to the above example:

6-Chloro-2,3-dimethyl-*N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-quinoxaline-5-carboxamide

5-Chloro-3-phenyl-*N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-3H-benzotriazole-4-carboxamide

6-Chloro-*N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-benzo[1,2,3]thiadiazole-7-carboxamide

5,6-Dichloro-*N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-1,3-dihydro-isobenzofuran-7-carboxamide

6-Chloro-2,3-dimethyl-*N*-[2-(*N*-formyl-*N*-hydroxy-amino)-ethyl]-2,3-dihydro-benzofuran-7-carboxamide

With appropriate manipulation and protection of any chemical functionality, synthesis of the remaining compounds of Formula (I) is accomplished by methods analogous to those above and to those described in the Experimental section.

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The present compounds are useful for the treatment of bacterial infections including but not limited to respiratory tract infections and/or Gram positive infections.

Compounds of Formula (I) and their pharmaceutically acceptable salts may be administered in a standard manner for antibiotics, for example orally, parenterally, sub-lingually, dermally, transdermally, rectally, via inhalation or via buccal administration.

Compositions of Formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as syrups, tablets, capsules, creams and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or

suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

Each dosage unit for oral administration contains suitably from 0.1 mg to 500 mg/Kg, and preferably from 1 mg to 100 mg/Kg, and each dosage unit for parenteral administration contains suitably from 0.1 mg to 100 mg/Kg, of a compound of Formula(I) or a pharmaceutically acceptable salt thereof calculated as the free acid. Each dosage unit for intranasal administration contains suitably 1-400 mg and preferably 10 to 200 mg per person. A topical formulation contains suitably 0.01 to 5.0% of a compound of Formula (I).

The daily dosage regimen for oral administration is suitably about 0.01 mg/Kg to 40 mg/Kg, of a compound of Formula(I) or a pharmaceutically acceptable salt thereof calculated as the free acid. The daily dosage regimen for parenteral

administration is suitably about 0.001 mg/Kg to 40 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free acid. the daily dosage regimen for intranasal administration and oral inhalation is suitably about 10 to about 500 mg/person. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit the desired activity.

No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the following test:

Biological Assay:

S. aureus or E. coli PDF activity is measured at 25°C, using a continuous enzyme-linked assay developed by Lazennec & Meinnel, (1997) "Formate dehydrogenase-coupled spectrophotometric assay of peptide deformylase" Anal. Biochem. 244, pp.180-182, with minor modifications. The reaction mixture is contained in 50 uL with 50 mM potassium phosphate buffer (pH7.6), 15 mM NAD, 0.25 U formate dehydrogenase. The substrate peptide, f-Met-Ala-Ser, is included at the K_M concentration. The reaction is triggered with the addition of 10 nM Def1 enzyme, and absorbance is monitored for 20 min at 340 nm.

Antimicrobial Activity Assay

Whole-cell antimicrobial activity was determined by broth microdilution using the National Committee for Clinical Laboratory Standards (NCCLS) recommended procedure, Document M7-A4, "Methods for Dilution Susceptibility Tests for Bacteria that Grow Aerobically" (incorporated by reference herein). The compound was tested in serial two-fold dilutions ranging from 0.06 to 64 mcg/ml. A panel of 12 strains were evaluated in the assay. This panel consisted of the following laboratory strains: *Staphylococcus aureus* Oxford, *Staphylococcus aureus* WCUH29, *Enterococcus faecalis* I, *Enterococcus faecalis* 7, *Haemophilus influenzae* Q1, *Haemophilus influenzae* NEMC1, *Moraxella catarrhalis* 1502, *Streptococcus pneumoniae* 1629, *Streptococcus pneumoniae* N1387, *Streptococcus*

pneumoniae N1387, *E. coli* 7623 (AcrABEFD+) and *E. coli* 120 (AcrAB-). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of compound that inhibited visible growth. A mirror reader was used to assist in determining the MIC endpoint.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the area can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.